

Proton secretion in rice leaves

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Abstract. Some properties of proton secretion by rice leaf segments were investigated. The influence of light on proton secretion was also examined. Proton secretion was found to be an active process. The inhibition by vanadate but not by nitrate and promotion by fusicoccin indicated that proton secretion by rice leaf segments originated from an ATP-driven H^+ pump located in the plasma membrane. KCl , $CaCl_2$ and $MgCl_2$ had stimulatory effect on proton secretion under both light and dark conditions. $CaCl_2$, in contrast, inhibited proton secretion. The relative order by which anions stimulated proton secretion in the dark was $KBr \geq KClO_3 > KNO_3 > KI > KCl$. No significant difference in stimulating proton secretion was observed among anions in the light. Light-stimulated proton secretion is detectable when the outer medium includes KCl , $NaCl$, KI and KNO_3 . In contrast, light inhibition of proton secretion was observed when leaf segments were incubated in the presence of $MgCl_2$ and KBr . Furthermore, light exerts no effect on proton secretion in the presence of $CaCl_2$ and $KClO_3$. It is likely that the influence of light on proton secretion depends on the assay condition. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea inhibited light-stimulated proton secretion suggesting that light-stimulated proton secretion is related to photosynthetic system. Proton secretion under both light and dark conditions was found to be promoted by benzyladenine but inhibited by indoleacetic acid, abscisic acid and cycloheximide.

Key words: Proton secretion; *Oryza sativa* L.

Introduction

Exchange of H^+ between plant cells and their surroundings is associated with a wide range of metabolic processes, and especially solute transport. It has been shown that plant tissues are capable of extruding H^+ into the outer medium (Bown, 1982; Bown and Nicholls, 1985; Gabella and Pilet, 1979; Gepstein, 1982; Lin, 1979; Van Volkenbrough and Cleland, 1980).

Abbreviation: ABA, abscisic acid; BA, benzyladenine; CHI, cycloheximide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Reports on the influence of light on proton secretion by leaves are controversial. In slices of oat, *Atirplex*, pea and bean leaves, light stimulated net proton efflux (Gepstein, 1982; Luttge *et al.*, 1970; Nobel, 1969; Van Volkenbrough and Cleland, 1980). In contrast, light was found to inhibit proton secretion from isolated *Asparagus* mesophyll cells (Bown, 1982; Bown and Nicholls, 1985).

In the present study, some characteristics of proton secretion by rice leaf segments were examined. The influence of light on proton secretion was also investigated.

Materials and Methods

Plant Material

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were cultured as previously described (Cheng and Kao, 1984). Briefly, seedlings were planted on a stainless net floating on half-strength Johnson's modified nutrient solution (Johnson et al., 1957) in a 500-ml beaker. The nutrient solution (pH 4.8) was refreshed every five days. Rice seedlings were grown in a green house with natural day light at 30°C day/25°C night. The apical 3-cm segments of the third leaves of 12-day-old seedlings were used for the measurement of proton secretion.

Measurements of Proton Secretion

The leaf segments were cut into 0.2 cm pieces. Four hundred and fifty 0.2 cm segments weighing 150 mg were incubated in 5 ml solution containing 250 mM mannitol and 1 mM KCl at 27°C and were shaken on a shaker at a speed of 70 cycles per minute. The light intensity used was 20 Wm⁻². For dark treatment, flasks containing leaf segments were wrapped in aluminum foil. Proton secretion from leaf segments was made by measuring the changes of the pH [$-\Delta\text{pH} = -(\text{final pH} - \text{initial pH})$] in the medium at frequent intervals or at 4 h after incubation. The initial pH was adjusted with 1 mM NaOH to pH 6.5 to 6.8.

Results

The effect of light on proton secretion by rice leaf segments is shown in Fig. 1. Light clearly resulted in the pH decrease of the medium containing KCl. Mannitol was found to amplify proton secretion. In the following studies, 250 mM mannitol was included unless otherwise indicated.

Most proton secretion assays were carried out in the presence of KCl. It would be of

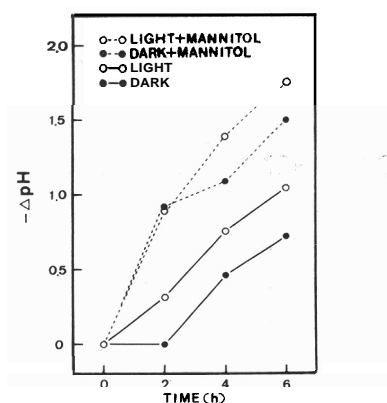


Fig. 1. Effect of mannitol on proton secretion in the light and in the dark.

great interest to investigate cation effects on the light stimulation of proton secretion. Figure 2 shows the proton secretion by leaf segments in the presence of KCl, NaCl, CaCl₂ and MgCl₂, respectively, under light and dark conditions. Proton secretion was found to be promoted by KCl, NaCl and MgCl₂ under light and dark conditions when compared with the control (mannitol only). However, CaCl₂ had inhibitory effect on proton secretion in the light and in the dark. Figure 2. also indicated that light-stimulated proton secretion was

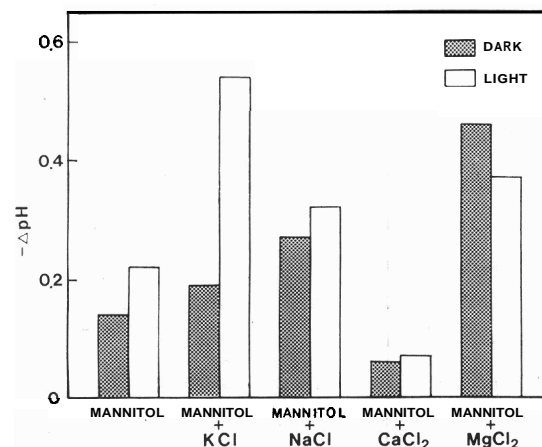


Fig. 2. Effect of cations on proton secretion in the light and in the dark. The cation concentration was 1 mM. Proton secretion was determined at 4 h after incubation.

observed in the presence of NaCl . In contrast, light inhibited proton secretion when leaf segments were in the presence of MgCl_2 . Light, on the other hand, exerted no promotive effect on proton secretion in the presence of CaCl_2 .

Figure 3. shows anion effect on the proton secretion by segments under light and dark conditions. No significant difference in proton secretion was observed among anions under light condition. However, proton secretion under dark condition was stimulated by the anions with the decreasing order: $\text{KBr} > \text{KClO}_3 > \text{KNO}_3 > \text{KI} > \text{KCl}$. It is interesting that proton secretion was inhibited by light when KBr was included in the medium. In addition, proton secretion by leaf segments into KClO_3 solution under light condition was similar to that in the dark.

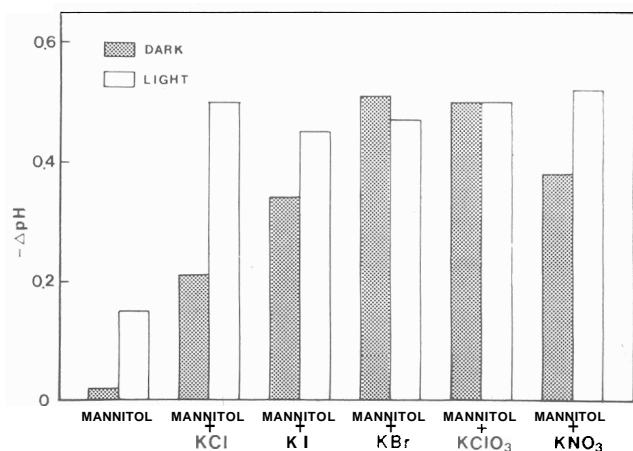


Fig. 3. Effect of anions on proton secretion in the light and in the dark. Anion concentration was 1mM . Proton secretion was determined at 4h after incubation.

The influence of photosynthesis on proton secretion was examined by the addition of DCMU. DCMU did not affect proton secretion in the dark (Fig. 4). However, DCMU significantly decreased proton secretion by leaf segments under illumination. These data

suggest that light-stimulated proton secretion is related to photosynthetic system.

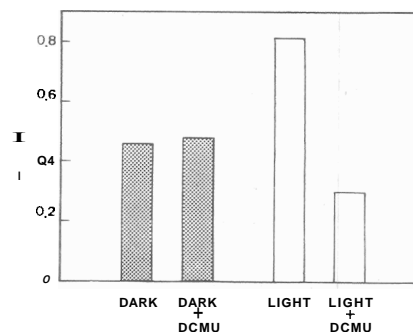


Fig. 4. Effect of DCMU on proton secretion by rice leaf segments. DCMU concentration was $10\ \mu\text{M}$. Proton secretion was determined at 4h after incubation.

Vanadate significantly depressed the acidification of the medium under dark and light conditions (Fig. 5). It is important to note that this effect cannot be due to its buffer capacity since the control includes phosphate buffer. Table 1 shows that fusicocin had strong effect in promoting proton secretion.

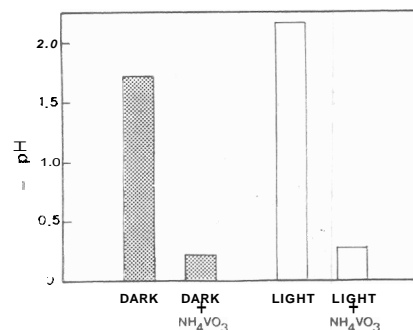


Fig. 5. Effect of vanadate on proton secretion by rice leaf segments. Vanadate concentration was 5mM . The solutions of light and dark controls contained 0.25mM sodium phosphate buffer, 5mM NH_4Cl , and 1mM KCl . Proton secretion was determined at 4h after incubation.

The effects of BA, CHI and ABA on proton secretion by leaf segment under light and dark conditions are shown in Fig. 6. Proton secretion

Table 1. Effect of fusicoccin on proton secretion by rice leaf segments under light and dark condition. Proton secretion was determined at 4h after incubation

Treatment	$-\Delta\text{pH}$
Light	1.06
Light+fusicoccin, 0.1 μm	2.02
Light+fusicoccin, 1 μm	2.63
Dark	0.83
Dark+fusicoccin, 0.1 μm	1.79
Dark+fusicoccin, 1 μm	2.54

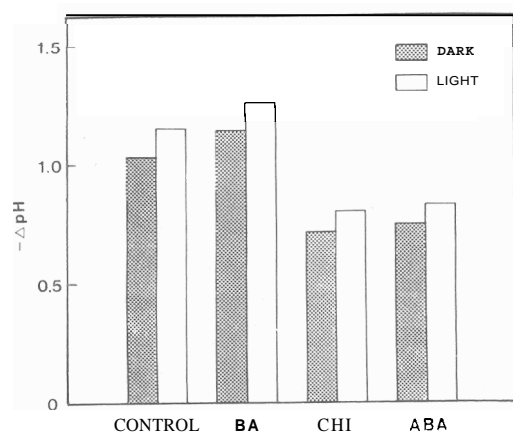


Fig. 6. Effect of CHI, BA and ABA on proton secretion in the light and in the dark. The concentration of BA and ABA was 10 μM , whereas that of CHI was 50 μM . Proton secretion was determined at 4h after incubation.

Table 2. Effect of indoleacetic acid on proton secretion by rice leaf segments under light and dark condition. Proton secretion was determined at 4h after incubation

Indoleacetic acid (mM)	$-\Delta\text{pH}$	
	Light	Dark
0	1.46	1.20
0.001	1.34	0.99
0.01	1.24	0.90
0.1	1.09	0.78

was found to be promoted by BA and inhibited by CHI and ABA. The stimulatory effect of

BA on proton secretion has been found in several experiments. The effect of BA, though slight, was therefore not an accidental event, but rather a true effect. Table 2 shows that indoleacetic acid significantly inhibited proton secretion by rice leaf segments under both light and dark conditions. The inhibition of proton secretion is increased with increasing of concentration of indoleacetic acid.

Discussion

Since the H^+ concentration of the outer medium (pH 6.5–6.8) was higher than that of the cytoplasm, which is considered to be a pH of about 7.0–7.5 (Smith and Raven, 1979), proton secretion by rice leaf segments as shown in our studies therefore was considered to be an active process. The changes in the rate of proton secretion reported here are unlikely due to accumulation of respiratory CO_2 or photosynthetic depletion of CO_2 , since leaf segments were vigorously shaken.

Vanadate is known to inhibit the plasma membrane located ATPase, whereas nitrate has an inhibitory effect on tonoplast-type and mitochondria H^+ -ATPases (Rea and Sanders, 1987; Sze, 1985). Fusicoccin is known to have an activating influence on plasma membrane ATPase (Marre, 1979). The results of vanadate sensitivity, no inhibition by nitrate and activating influence of fusicoccin strongly support the conclusion that proton secretion by rice leaf segments originated from ATP-driven H^+ pump located in the plasma membrane.

Proton secretion by rice segments is unlikely via an H^+/K^+ antiport as suggested by Lin (1979) in corn roots, since not only K^+ but also Na^+ and Mg^{2+} had a significant effect on the net release of H^+ .

Proton secretion assays are commonly carried out in the presence of KCl. From our results, it is suggested that an optimum assay

should include KBr or KClO_3 to manifest both cation and anion effects. Our result also shows that proton secretion was enhanced by the presence of mannitol. Gepstein (1982) suggested that the amplification of proton secretion by mannitol was attributed to the reduction of turgor pressure which might affect the activity of a plasma membrane H^+ -ATPase.

The inhibition of proton secretion by CHI seems to suggest that protein synthesis is required for proton secretion. It has long been recognized that plant hormones play an important role in the regulation of ion transport (Van Steveninck, 1976). We found that BA promoted and ABA inhibited proton secretion by rice leaf segments. Indoleacetic acid is known to promote proton secretion by coleoptile and hypocotyl segments (Van Steveninck, 1976). However, we found that proton secretion by rice leaf segments was significantly inhibited by indoleacetic acid. It seems that the effect of indoleacetic acid on proton secretion is tissue specific. It remains to be elucidated whether BA, indoleacetic acid and ABA affect proton secretion via direct effect on the activity of a plasma membrane H^+ -ATPase or metabolic processes which involve the establishment of proton gradients.

Light has been shown to stimulate proton secretion by oat, *Atriplex*, pea and bean leaves but inhibit proton secretion from isolated *Asparagus* mesophyll cells (Bown, 1982; Bown and Nicholls, 1985; Gepstein, 1982; Luttge *et al.*, 1970; Nobel, 1969; Van Volkenbourgh and Cleland, 1980). In this study, we found that light-stimulated proton secretion is detectable when the outer medium includes solutions of KCl, KI, NaCl and KNO_3 . In contrast, light was observed to inhibit proton secretion in the presence of MgCl_2 and KBr. It appears that the influence of light on proton secretion

depends on the assay condition.

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水稻葉片氫離子釋放的研究

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本研究主要之目的在於瞭解水稻葉片氫離子釋放之一些特性與光線對氫離子釋放之影響。供試品種為臺中在來一號。水稻葉片氫離子釋放是一種主動過程 (active process) 鈣鹽酸抑制水稻葉片氫離子之釋放，但硝酸鹽却無抑制氫離子釋放之作用。同時，fusicoocin 促進氫離子釋放。由此特性推測水稻葉片氫離子之釋放係受細胞膜上 ATP 所推動之氫離子泵 (H^+ pump) 之控制。KCl, NaCl, $MgCl_2$ 均可促進光線與黑暗下氫離子之釋放。然而， $CaCl_2$ 則抑制氫離子之釋放。不同陰離子（鉀鹽）於光照下對氫離子之釋放並無明顯之差異。在黑暗下，陰離子促進氫離子釋放之能力，依序為 $KBr \geq KClO_3 > KNO_3 > KI > KCl$ 。Cycloheximide 與 abscisic acid 可抑制光線與黑暗下氫離子之釋放，而 benzyladenine 則促進光線與黑暗下氫離子之釋放。當葉片漂浮於 KCl, NaCl, KI 與 KNO_3 溶液時，光線可促進氫離子之釋放至溶液內。然而，葉片漂浮於 $MgCl_2$ 與 KBr 溶液時，光線抑制氫離子之釋放。再者，葉片如漂浮於 $CaCl_2$ 與 $KClO_3$ 溶液時，光線與黑暗處理對氫離子之釋放則無明顯之差異。因此，光線對氫離子釋放之影響似乎決定於葉片漂浮在何種溶液。DCMU 可抑制光線所促進之氫離子釋放，此顯示，光線促進之氫離子釋放可能與光合作用系統有關。